TITLE: LONGITUDINAL ANALYSIS OF PULMONARY MICROBIOME IN CYSTIC FIBROSIS PATIENTS BY CULTURE DEPENDENT AND CULTURE INDEPENDENT METHODS: EXPERIENCE AT A BRAZILIAN CENTRE

AUTHORS: LEITE, C.C.F.[1]; FREITAS, F.A.D.[1]; FIRMIDA, M.C.[2]; SILVA, E.A.S.R.[1]; LEÃO, R.L.[1] ; ALBANO, R.M.[3], MARQUES, E.A.[1]

INSTITUTION: [1]Universidade do Estado do Rio de Janeiro Departamento de Microbiologia, Imunologia e Parasitologia; [2]Departamento de Doenças do Tórax; [3]Departamento de Bioquímica

ABSTRACT:

Repeated episodes of pulmonary exacerbations and progressive deterioration of lung functions are intrinsic of Cystic Fibrosis (CF) and the leading causes of mortality among this patient population. Although the negative impact of infection in CF patients and importance of respiratory samples cultures are fully recognized, culture-independent molecular methods came with the promise to expand our understanding of CF airway microbiology. We performed a prospective study in a CF centre for adults in Rio de Janeiro, Brazil, from June 2014 to July 2015. Clinical and microbiological data (obtained by standard culture dependent methods) from five patients was collected over one year of study and microbiome analysis of 25 respiratory secretions was performed by sequencing the V4 region of the 16S rRNA gene in an Illumina MiSeq® Next Generation Sequencing instrument. The resulting sequence data were analysed using software MOTHUR® regarding diversity and relative abundance of operational taxonomic units (OTUs).The lung microbiome analysis of all samples revealed 190 OTUs, from which 51 were present in more than one patient and 64 were >1% in frequency. The most abundant genera was *Staphylococcus*, represented by 32,9 % of the OTUs, followed by *Burkholderia* (11,4%) *Pseudomonas* (10,4%) and *Fusobacterium* (7,4%). Longitudinal evaluation of each patient´s lung microbiome was performed. The diversity and relative abundance of each OTU was examined longitudinally and two patients showed microbial communities dominated by *Burkholderia* (71,2% of the OTUs over one year) and *Staphylococcus* (87,5% of the OTUs over one year). Based in the classic microbiology method, those patients were chronic colonized by *Burkholderia vietnamiensis* and *Staphylococcus aureus*, respectively. The three other patients showed greater diversity of genera - including those representing the classic pathogens in CF, anaerobes and commensals - with quantitative fluctuations of diversity and relative abundance of OTUs among the study period. When comparing the culture dependent to the culture independent methods, both methodologies revealed the presence of the essentially known classic pathogens in CF. Our results suggest that lung microbiome in CF is highly patient-specific and that it should be investigated as such.

KEYWORDS: CYSTIC FIBROSIS, MICROBIOME, METAGENOMIC ANALYSIS

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